UNIFORMITY IN INVERTASE ACTION. II

DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF PURE SCIENCE, COLUMBIA UNIVERSITY

BY

FRANKLIN HOLLANDER

NEW YORK CITY
1923

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UNCLASSED
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TO MY PARENTS

ACKNOWLEDGMENT.

The author wishes to express to Professor John M. Nelson his gratitude for the inspiration and encouragement which he has given him during this work.

UNIFORMITY IN INVERTASE ACTION.

In their paper on "Uniformity in invertase action," Nelson and Hitchcock (1) found that six out of eight preparations of invertase studied, showed a uniform behavior with respect to the rate of inversion of a 10 per cent sucrose solution. In other words, the hydrolysis in each case conformed to the same empirical equation, involving time and per cent hydrolyzed. These six were Preparations 1, 2, 7, 8, A, and B. The remaining two, Nos. 3 and 6, showed a progressive decrease in volocity. Not only did these two differ from the majority but also from each other, and because of their apparent irregularity the authors termed them "abnormal" preparations.

A similar case of abnormality has been described recently by Willstätter, Graser, and Kuhn (2). They observed that all but one of several very active preparations gave the usual increasing monomolecular velocity constants during the course of the hydrolysis. This one, Preparation 1, gave practically constant coefficients under the same conditions.

Nelson and Hitchcock, and also Willstätter and his associates were of the opinion that the abnormality could hardly be due to an inactivation[1] of the enzyme occurring during the process of hydrolysis. The basis for this conclusion was the same in both cases: when the abnormal invertase preparation was permitted to remain in the constant temperature bath for the same length of time as that over which the hydrolysis was followed, no appreciable loss in activity was found to have occurred.

[1] The term inactivation as used here refers specifically to the progressive loss of activity which invertase manifests on standing, especially at higher temperatures and acidities.

These experiments, however, do not take into account the possible influence of the hydrogen ion concentration on the rate of inactivation. That this factor is an extremely important one has been shown by O'Sullivan and Tompson (3), Hudson and Paine (4), and by von Euler and Laurin (5). Due to this omission the conclusion reached, that abnormality could not be due to inactivation, becomes questionable. The present paper, therefore, deals chiefly with a reexamination of this point.

The attempted inactivation of Preparation 3 by Nelson and Hitchcock was carried out at pH 5.8, without the addition of a buffer mixture. Since the abnormality was observed in the course of a hydrolysis at pH 4.5, it seemed more logical to the present investigators to look for inactivation in the neighborhood of this latter acidity. Under these conditions, a loss of activity was actually found to occur. In addition to Preparation 3, Nos. 6b, 7, and 8 were also studied; the two latter being normal preparations as shown by Nelson and Hitchcock. Unfortunately, none of No. 6 (one of the two original abnormal preparations of these authors) was available, and so No. 6b, No. 6 which had been dialyzed, was used instead of it.

Briefly, the procedure employed was as follows: Enough of a citrate buffer solution yielding the desired pH was added to the enzyme solution so as to be present in 0.01 M concentration. At the end of each of the indicated time intervals after mixing, a sample of this incubation mixture was withdrawn and added to a sucrose-buffer solution, such that the resulting hydrolysis mixture contained 10 per cent sucrose and buffer in 0.01 M concentration at a pH of about 4.8 at 25°C. Under these conditions the empirical equation of Nelson and Hitchcock

$$n.t = \frac{\log 100}{(100 - p)} + 0.002642\,p - 0.00000886\,p^2 - 0.0000001034\,p^3$$

is applicable. Taking three samples during the course of each hydrolysis, it is possible to calculate n, the velocity coefficient in the above relation. In this way, the activity at any time is determined as the average of three values of n, thus increasing the certainty of the results. The experimental details are given in the next section. Duplicate experiments were entirely independent.

Table I contains a summary of these experiments; the more detailed data are given in Table VI. As was anticipated, the two normal preparations show no decrease in n and, therefore, no loss of activity whatever during the period of incubation. Nos. 3 and 6b, however, show decreases of 1.1 and 2.7 per cent, respectively, during the 5 hour period, the length of time over

TABLE I.

Experiment No.	Invertase No.	Inactivation time.	Varied condition.	$n \times 10^5$	Inactivated.
		hrs.	*pH*		*per cent*
18a	7	0	4.8	823	
b		5		820	0.4
2b	8	2	4.8	988	
d		6		988	0.0
69a	3	0	4.9	840	
b		5		831	1.1
70a	3	0	4.9	840	
b		5		831	1.1
71a	6B	0	4.9	72.7	
b		5		70.7	2.7
72a	6B	0	4.9	72.7	
b		5		70.7	2.7
63a	3	0	+Invert sugar.	801	
b		5	4.9	786	1.9
64a	3	0	+Invert sugar.	802	
b		5	4.9	787	1.9

which the original abnormal hydrolyses were followed. Such an inactivation occurring during the course of a hydrolysis would obviously manifest itself by progressively decreasing velocity constants. It has been claimed by O'Sullivan and Tompson (3) and by Hudson and Paine (4) that both sucrose and fructose can exert a protective action on this inactivation, although its extent varies with the pH and the concentration of added carbohydrate. In view of this it becomes necessary to show that the sugars present during the hydrolysis are not able completely

to stabilize Preparation 3 over this period. Otherwise there remains the possibility of the observed decrease in n being due to some other cause, in spite of the inactivation which occurs in the absence of the sugars. Accordingly, Experiments 69 and 70 (Table I) were repeated with this modification, that invert sugar was present in the buffer solution before the invertase was added to give the incubation mixture. The concentration of invert sugar after completed inversion was approximately the same as

TABLE II.

Experiment No.	Invertase No.	Inactivation time.	Varied conditions.	$n \times 10^5$	Inactivation.
		hrs.			*per cent*
22a	3	0	+Boiled preparation No. 8	856	0.0
b		5		856	0.0
47a	6B	0	+Boiled preparation No. 8.	76.2	
b		5		74.1	2.8
48a	6B	0	+Boiled preparation No. 8.	76.0	
b		5		74.1	2.5
23a	3	0	+0.02 M NaCl.	790	
b		5		791	0.0
43a	6B	0	+0.1 M NaCl.	75.8	
b		5		72.6	4.2
44a	6B	0	+0.1 M NaCl.	76.1	
b		5		72.4	4.9

that occurring in the hydrolyses described by Nelson and Hitchcock. A comparison of Experiments 63 and 64 with Nos. 69 and 70 indicates the absence of any such protective action.

With respect to the influence of added substances, Nelson and Hitchcock found that they were able to vary the degree of abnormality in the following ways: (1) by the addition of invertase which had been inactivated by boiling, and (2) by the addition of sodium chloride. If, now, inactivation is the cause of abnormality, then it should be similarly affected by these substances. This was found to be so. The results of these experiments are given in Table II while Table III contains a summary of the

observations of Nelson and Hitchcock. It must be remembered here that 0.7 per cent was arbitrarily fixed by these investigators as a boundary value of the A. D.[2] for the distinction of normal and abnormal preparations.

The procedure was the same as that described above except that in each case the indicated stabilizing agent was mixed with the buffer solution before the addition of the invertase. The inactivation of the added yeast extract was accomplished as

TABLE III.

A Summary of Nelson and Hitchcock's Experiments with Abnormal Invertase.

Experiment No. (Nelson and Hitchcock).	Invertase preparation No.	Invertase per 100 cc. of solution.	Varied condition.	A.D.
		cc.		*per cent*
12	3	1.905	None.	1.4
17	6	10.45	"	1.9
20	7	3.60	"	0.56*
22	6B	16	Dialyzed and diluted.	1.8
25	3	1.905	+10 cc. of boiled invertase 8.	0.34*
26	3	1.943	+10 " " " " 8.	0.40
35	6	10.45	+10 " " " " 8.	1.4
44	3	1.905	+0.02 M NaCl.	0.71*
42	3	1.905	+0.1 " "	0.46*
48	6	10.45	+0.1 " "	1.7
58	3	0.5	Concentration of invertase.	2.7
54	3	3.0	" " "	0.74
56	3	6.0	" " "	0.67*

*These hydrolyses are normal since the boundary A.D. value set for the distinction of normal and abnormal preparations by Nelson and Hitchcock is 0.70 per cent.

described by Nelson and Hitchcock; namely, by boiling until the solution no longer showed any hydrolyzing power, the loss of water by evaporation being corrected for before using.

It is evident from Experiment 22 (Table II) that Preparation 3, which was normalized by the addition of 10 cc. of boiled No. 8 (Nelson and Hitchcock, Experiments 25 and 26), is also completely stabilized by it during the 5 hour incubation period. On the

[2] A. D. has its usual significance in the theory of errors; it represents the average percentage deviation from the mean of the series of n values for any one hydrolysis.

other hand, with Preparation 6 which was but slightly, if at all, normalized (Nelson and Hitchcock, Experiment 35), there was a corresponding failure of protection against inactivation as shown by Experiments 47 and 48, although they were performed with the enzyme solution after further dialysis. Exactly the same parallelism between the two phenomena occurs with respect to the influence of salt, as becomes evident from a comparison of Experiments 42 and 44 (Table III) with No. 23 (Table II) for Preparation 3, and Experiment 48 (Table III) with Nos. 43 and 44 (Table II) for No. 6.

An observation similar to the above has been reported by von Euler and Laurin (5) who found that a boiled invertase solution could inhibit the inactivation of an unboiled one from the same source. But when the influence of an inactivated bottom yeast extract on a solution from top yeast was investigated, it was found to be nil. With respect to the stabilizing action of sodium chloride on invertase, no reference could be found in the literature, although Willstätter and his coworkers (2) have observed such an effect with calcium chloride. Amylase and ptyalin, however, have been investigated with respect to sodium chloride protection by Ernström (6). While salt had a very marked stabilizing action on the latter, so high a concentration as 0.09 M had no appreciable influence on the former. In the case of ptyalin, the effect increases rapidly with concentration of sodium chloride to a maximum at 0.1 M and then falls off slowly. That this distinction between the two enzymes is fundamental is open to question, in view of the fact that different preparations of the same enzyme can, as shown here with invertase, react differently in this respect.

In addition to the normalizing action exerted by sodium chloride and by yeast gum, the variation of degree of abnormality with enzyme concentration was also determined by Nelson and Hitchcock. Examination of the results of their Experiments 58, 12, 54, and 56 reveals the fact that the magnitude of the A. D. decreases with increased concentration of invertase. The values range from 2.7 per cent with 0.5 cc. of enzyme solution per 100 cc. of hydrolyzing mixture, to 0.67 per cent with 6 cc. per 100 cc. In the latter case, the A. D. is actually reduced to a value less than that set for the distinction of normal and abnormal prep-

arations. From the inactivation point of view, this is just the effect that would be predicted. The smaller the concentration

TABLE IV.

Experiment No.	Invertase No.	Inactivation time.	Varied condition.	$n \times 10^6$	Inactivated.
		hrs.	*pH*		*per cent*
51a	3	0	No Buffer.	855	
b		5	5.8	854	0.0
61a	3	0	5.8	862	
b		5		863	0.0
62a	3	0	5.8	862	
b		5		861	0.0
52a	6B	0	5.8	70.9	
b		5		61.9	12.7
53a	6B	0	5.8	70.8	
b		5		61.7	12.8
54a	7	0	5.8	523	
b		5		522	0.0
3a	8	2	5.8	983	
b		6		983	0.0
59a	7	0	2.4	524	
b		5		448	14.5
60a	7	0	2.4	522	
b		5		447	14.4
35a	8	0	2.4	1,190	
b		5		1,140	4.2
39a	6B	0	2.4	67.3	
b		5		42.2	37.3
40a	6B	0	2.4	67.3	
b		5		42.3	37.2

of enzyme, the greater is the time required for attainment of, say, 90 per cent inversion of the sucrose. But, since the inactivation is progressive, the loss of activity in the course of the

hydrolysis will also increase, and consequently the observed A. D. over these varying periods will grow larger as the dilution of invertase increases.

A comparison of Experiments 70 and 69 with the incubation experiment of Nelson and Hitchcock, referred to in the introduction to this paper, shows that, whereas invertase Preparation 3 loses none of its activity in 5 hours at pH 5.8, at 4.9 it loses 1.1 per cent. Unless the former stability was due to a difference in source of acidity, such as nature of buffer, this difference would indicate that the stability optimum of Preparation 3 is at a more alkaline value than 4.5, the value reported by von Euler and Laurin. Accordingly, after checking the observation of Nelson and Hitchcock (Experiment 51, Table IV), the incubation was repeated at the same pH in the presence of 0.01 M citrate buffer. As can be seen from the results of Experiments 61 and 62, the

TABLE V.

Experiments 12′ and 12″.

Spontaneous Activation of Invertase Hb—pH 3.3.

Experiment No.	Inactivation time.	$n \times 10^4$ (average).	Activated.
	hrs.		*per cent*
a	0	150	0
b	3.5	150	0
c	17	150	0
d	40.5	150	0
e	74	153	2
f	118	151	0.7

added buffer has no appreciable influence. It therefore follows that for Preparation 3 at 25°C., the stability optimum occurs at a pH greater than 4.5 and in the neighborhood of 5.8, furnished by citrate buffer.

Regarding the other three enzyme solutions, the results of inactivations at both pH 6.6 and 2.4 (Table IV) suggest that under these conditions the stability optima of these preparations are also higher than pH 4.5. In the case of No. 6b, the losses at 6.6, 4.9, and 2.4 are 12.8, 2.7, and 37.2 per cent, respectively, the first being about one-third of the last. Similarly, the two normal invertases are stable at both the lower hydrogen ion concentrations while at pH 2.4 the losses are 4.2 and 14.4 per cent for Nos. 8 and 7, respectively.

TABLE VI.

t equals time of sampling in minutes.

Experiment 2b.		Experiment 2d.		Experiment 12′e.		Experiment 12″e.	
t	$n \times 10^5$	t	$n \times 10^5$	t	$n \times 10^5$	t	$n \times 10^5$
25	991	25	990	25.05	1,538	20	1,532
30	988	30	987	35	1,528	30	1,525
35	985	35	987	45	1,530	40	1,524
Mean....	988	Mean....	988	Mean....	1,532	Mean....	1,527
Experiment 3a.		Experiment 3b.		Experiment 12′f.		Experiment 12″f.	
t	$n \times 10^5$	t	$n \times 10^5$	t	$n \times 10^5$	t	$n \times 10^5$
25	979	25	979	25	1,506	20	1,512
30	983	30	985	35	1,506	30	1,507
35	986	35	986	45	1,509	40	1,506
Mean....	983	Mean....	983	Mean....	1,507	Mean....	1,508
Experiment 12′a.		Experiment 12″a.		Experiment 18a.		Experiment 18b.	
t	$n \times 10^5$	t	$n \times 10^5$	t	$n \times 10^5$	t	$n \times 10^5$
19.23	1,509	16	1,513	25	827	25	824
29.1	1,499	26	1,499	30	822	30	819
40.0	1,503	34	1,494	35.1	821	35.1	817
Mean....	1,504	Mean....	1,502	Mean....	823	Mean...	820
Experiment 12′b.		Experiment 12″b.		Experiment 22a.		Experiment 22b.	
t	$n \times 10^5$	t	$n \times 10^5$	t	$n \times 10^5$	t	$n \times 10^5$
20	1,510	15.17	1,512	39	856	39	857
30.17	1,498	25.4	1,506	55	858	55	858
40.07	1,500	35.33	1,504	70	854	70	852
Mean..	1,503	Mean....	1,507	Mean....	856	Mean....	856
Experiment 12′c.		Experiment 12″c.		Experiment 23a.		Experiment 23b.	
t	$n \times 10^5$	t	$n \times 10^5$	t	$n \times 10^5$	t	$n \times 10^5$
20	1,504	15.08	1,506	39	791	39	790
30	1,498	25	1,488	55	791	55	794
40	1,497	35	1,488	70	787	70	788
Mean....	1,500	Mean....	1,494	Mean. ..	790	Mean....	791
Experiment 12′d.		Experiment 12″d.		Experiment 35a.		Experiment 35b.	
t	$n \times 10^5$	t	$n \times 10^5$	t	$n \times 10^4$	t	$n \times 10^4$
30	1,505	25.25	1,502	15	119	15	114
40.13	1,500	35	1,494	20	118	20	114
50.25	1,501	45	1,497	25	119	25	114
Mean	1,502	Mean....	1,498	Mean ...	119	Mean....	114

TABLE VI—*Continued.*

Experiment 39a.		Experiment 39b.		Experiment 51a.		Experiment 51b.	
t	$n \times 10^6$	t	$n \times 10^6$	t	$n \times 10^5$	t	$n \times 10^5$
120	672	120	424	40	858	40	856
150	671	150	422	55	854	55	854
192.3	677	180.2	420	70.1	854	70.1	852
Mean....	673	Mean....	422	Mean....	855	Mean....	854
Experiment 40a.		Experiment 40b.		Experiment 52a.		Experiment 52b.	
t	$n \times 10^6$	t	$n \times 10^6$	t	$n \times 10^5$	t	$n \times 10^6$
120	672	120	424	119.9	715	119.9	623
150	668	150	422	150	707	150	617
168	680	180	424	180	706	180	618
Mean....	673	Mean....	423	Mean....	709	Mean....	619
Experiment 43a.		Experiment 43b.		Experiment 53a.		Experiment 53b.	
t	$n \times 10^6$	t	$n \times 10^6$	t	$n \times 10^6$	t	$n \times 10^6$
119.1	764	120	731	120	716	120	623
150	759	150	725	150	701	150	612
180	750	180	723	180	708	180	616
Mean....	758	Mean....	726	Mean ...	708	Mean....	617
Experiment 44a.		Experiment 44b.		Experiment 54a.		Experiment 54b.	
t	$n \times 10^6$	t	$n \times 10^6$	t	$n \times 10^5$	t	$n \times 10^5$
120	768	120	729	30	522	30	521
150	761	150	723	35	522	35	523
180	754	180	721	40	524	40	522
Mean ...	761	Mean....	724	Mean...	523	Mean...	522
Experiment 47a.		Experiment 47b.		Experiment 59a.		Experiment 59b.	
t	$n \times 10^6$	t	$n \times 10^6$	t	$n \times 10^5$	t	$n \times 10^5$
120	767	120	750	30	523	30	446
150	761	150	740	35	524	35	447
180	758	180.1	733	40	526	40	450
Mean	762	Mean....	741	Mean ...	524	Mean....	448
Experiment 48a.		Experiment 48b.		Experiment 60a.		Experiment 60b.	
t	$n \times 10^6$	t	$n \times 10^6$	t	$n \times 10^5$	t	$n \times 10^6$
120	765	120	748	30	521	30	444
150	761	150	739	35	521	35	447
180.1	754	180	737	40	523	40	449
Mean	760	Mean....	741	Mean ...	522	Mean....	447

TABLE VI—*Concluded.*

Experiment 61a.		Experiment 61b.		Experiment 69a.		Experiment 69b.	
t	$n \times 10^5$	t	$n \times 10^5$	t	$n \times 10^5$	t	$n \times 10^5$
40	863	40	866	41	842	40.5	829
55	863	55	862	55	838	55	833
70	862	70	862	70	839	70	832
Mean....	862	Mean...	863	Mean....	840	Mean....	831
Experiment 62a.		Experiment 62b.		Experiment 70a.		Experiment 70b.	
t	$n \times 10^5$	t	$n \times 10^5$	t	$n \times 10^5$	t	$n \times 10^5$
40	861	40	862	40	843	40	823
55	864	55	863	55	838	55	831
70	862	70	858	70.5	840	70	830
Mean	862	Mean....	861	Mean ...	840	Mean ...	831
Experiment 63a.		Experiment 63b.		Experiment 71a.		Experiment 71b.	
t	$n \times 10^5$	t	$n \times 10^5$	t	$n \times 10^6$	t	$n \times 10^6$
40	806	40	791	120	728	120	709
54	801	55	784	150	724	150	706
70	797	70	784	180	729	180	705
Mean....	801	Mean ...	786	Mean ...	727	Mean....	707
Experiment 64a.		Experiment 64b.		Experiment 72a.		Experiment 72b.	
t	$n \times 10^5$	t	$n \times 10^5$	t	$n \times 10^6$	t	$n \times 10^6$
40	806	40	791	120	728	120	709
55	803	55	787	150	727	150	708
70	798	70	782	180	727	180	705
Mean ...	802	Mean....	787	Mean....	727	Mean....	707

This is in accord with the observation of Bloomfield (7), that the values for n decrease during a hydrolysis in the vicinity of pH 2, while at 4.5 and 7, they remain constant. Because of the small amounts of these various preparations available, it has not been possible to examine this point in greater detail.

As a matter of record, the following observations are also reported. In studying the relative stabilities at pH 2.4, invertase No. IIb, a new preparation made as described below, was found to have undergone no inactivation in 3½ hours (Experi-

ments 12′ and 12″, Table V). Continuing the incubations, the activity persisted unchanged for $40\frac{1}{2}$ hours, but after the 3rd day (74 hours) showed an increase of 2 per cent. This was followed 44 hours later by a drop to 0.7 per cent above the original value. The possibility of this being due to experimental error is eliminated by its magnitude as well as by the duplication of effect. At the same time the 74 hour samples were withdrawn from their respective incubation bottles, it was noticed that a fine white sediment had settled out in each of them.

EXPERIMENTAL DETAILS.

Procedure.—For studying the inactivation, the following solutions were prepared: An incubation mixture consisting of

(A) 1. Stock invertase solution,
2. Buffer solution of such a nature as to give the desired pH, and in such quantity as to yield a concentration of buffer salts equal to 0.01 molar.

(C) A solution consisting of
1. Sucrose,
2. Buffer combination,
3. Water in which these components were present in such proportions that when a convenient number of cc. of (A) was added to a given volume of (C), the hydrolyzing mixture (C′) contains the components in the following concentrations:
(a) Sucrose, 10 gm. per 100 cc.,
(b) Buffer, 0.01 molar, of pH 4.5,
(c) Invertase solution, in such an amount as to give a convenient velocity of hydrolysis.

Throughout the incubation, Solution (A) was kept in the thermostat. In order to follow the progress of the reaction, samples were pipetted out and added to Solution (C) immediately (within 1 minute) after mixing the components and at desired time intervals thereafter. The mean delivery time of the pipette was taken as the time of observation in all cases.

In order to determine the extent of hydrolysis at any time, a 25 cc. portion of (C′) was added to 5 cc. of 0.1 M sodium carbonate solution, which stopped the reaction. From $\frac{1}{4}$ to 2 hours after this, as recommended by Hudson (8), the polariscopic rotations of these solutions were determined. The initial rotation of the hydrolysis mixture was determined by adding a proportionate amount of Solution (A) to the sodium carbonate solution, in order to inactivate the enzyme. Then enough of (C) was added to this so as to make the relative amounts of the three components the same as in any other hydrolysis sample. The final readings were taken 2 or more days after the experiment was performed.

In order to get, as accurate as possible, a determination of the activity of Solution (A), the pH of (C′) was always kept at the optimum value, 4.5, thus giving a maximum value to the velocity constant hydrolysis n for the given invertase concentration. The relative amounts of the buffer components in Solution (C) necessary to give this value of pH, were determined as follows: The amounts of each of these components already present in the portion of Solution (A) added to Solution (C) were subtracted from the corresponding values for the resulting Solution (C′) employed in the hydrolysis experiment. When (A) contained more acid than was required in Solution (C′) then enough sodium hydroxide was added to neutralize the excess. In an inactivation experiment run over several months, a pH determination repeated after a long interval of time showed no change in the acidity. Such an experiment required the presence of toluene in Solution (A) to avoid growth of mold.

A description of the method of preparation used for all of the invertase solutions with the exception of No. Hb, is given in the paper of Nelson and Hitchcock. The procedure used was essentially that of Nelson and Born (9). In the case of the new preparation, No. Hb, the procedure was practically that of von Euler. The autolyzed bottom yeast juice was precipitated with an equal volume of 95 per cent alcohol, the gum taken up with distilled water and reprecipitated. An aqueous solution of the gum so obtained was used without being dialyzed.

The pH was determined colorimetrically, using the necessary Clark and Lubs indicators (10). The citrate standards used were calibrated electrometrically with bubbling hydrogen electrode against a saturated potassium chloride calomel cell, joined by a saturated potassium chloride bridge. The calculations were based on 0.1 N hydrochloric acid as a standard, its conductance dissociation being taken as 92.04 per cent at 25°C., at which temperature all determinations were made.

The light source used in the polariscopic work was a mercury vapor arc.

SUMMARY.

1. An explanation based on their lower relative stability has been offered for the "abnormality" of certain invertase preparations observed by Nelson and Hitchcock and by Willstätter and his coworkers.

2. It has been shown that sodium chloride and yeast gum exert a stabilizing influence on one, but not on the other, of the two abnormal preparations studied.

3. Data were obtained at 25°C. showing that the stability optimum of one abnormal invertase preparation did not occur in the pH region of optimum activity as suggested by von Euler and Laurin for the temperature interval 45–55°, but further towards the alkaline side; *i.e.*, in the vicinity of pH 5.8.

4. Likewise, data obtained with the two normal preparations suggest that they also do not agree with von Euler and Laurin's conclusion in this respect.

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